

LARVICIDAL AND GROWTH REGULATORY OF GUM RESIN POWDERS FROM ALOE VERA, ASTRAGALUS SARCOCOLLA, COMMEFORA MYRRHA AND FERULA ASSA-FOETIDA L AGAINST TROGODERMA GRANARIUM EVERTS

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Abstract

The Persistence Of The Toxicity And Growth Regulatory Effects Of The Gum Resin Powders Of Aloe Vera, Astragalus Sarcocolla, Commefora Myrrha And F. Assa-Foetida Were Tested Against Fifth Instar Trogoderma Granarium Everts Larvae. The Concentrations 1, 0.1, 0.3 And 0.5% G/G Of Each The Gum Resin Powders Were Mixed With 30 G Of Wheat Grains. As Expected, The 1% Concentration For A. Sarcocolla, Which Was The Highest Of All The Treatments, Caused 1.7, 3.3 And 16.7% Mortality At 10 Day Of Treatment For F. Assa-Foetida, A. Vera, And C. Myrrha, Respectively. All The Treatments Exhibited Larvicidal And Pupicidal Efficiency At 100% At 35 Day Of Treatment, Except For The 1% And 0.1% Concentrations Of The F Assa-Foetida Gum Resin Powder, Which Had Pupicidal Activity At 35% Mortality And Inhibited Pupation By 93.3% And At 35 Days Of Treatment Compared To The 98% Success Of Larvae Pupation In The Control Treatment At 10 Day Of Observation. All Treatments Led To Longevity In The 5th Instar Larvae For A Period Of More Than 25 Days Compared To The Larvae In The Control Treatment. There Was An Abnormality In The Shape, Sluggishness, Behavioural And Neurological Disorders In The Larvae Treated.

Keywords: *Trogoderma Granarium, Growth Regulatory, Late Pupation & Ferula Assa-Foetida*

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INTRODUCTION

Trogoderma granarium Everts, which belongs to the Coleoptera order and the Dermestidae family, is considered to be one of the most devastating pests of whole and ground cereals (Banks 1977).

This pest reduces the cooking quality of cereals and reduces their nutritional value. *T. granarium* is widely spread and feeds on various types of grains and dry animal products that cause damage to the grain germs (Jood et al., 1993) that reduces germination of the seeds infected with this pest (Khare et al., 1974). Morison (1925) reported on the health risks of the barbed hairs and the moulting skin that fall from the bodies of larvae. Parashar (2006) reported that, the 5th instar larvae of the Khobra beetle is the greediest instar in terms of stored products. According to Kerr (1988), the larvae will be able to live without food for more than thirty-three months, which makes it more difficult to control this pest. Its presence on food crops and the use of chemical pesticides leads to pollution, and repeated use would lead to resistance against these pesticides (Boyer et al., 2012; White, 1995).

Price and Mills (1988), Singh et al (1990) and Herrman (2006) revealed that there is unorganized and dense use of methyl bromide gas as a pesticide for combating *T. granarium* stages, which has been shown to be a

carcinogen in humans and has environmental risks. The use of some indigenous plant products against *Trogoderma granarium* as insecticides has been shown to provide effective protection (Al-Moajel, 2004; Jood et al., 1996; Sultana et al, 2016).

Some plants were proven to have insecticidal properties. The objective of this study is to assess the toxicity of some of the gum resins of the traditional medicine plants on the 5th instar larvae of the *Trogoderma granarium* Everts beetle, its pupation and the emergence of the adult pupae. These gum resins are frequently used in traditional or popular medicine in Asia and in Saudi Arabia.

MATERIALS AND METHODS

Breeding the Insects

To obtain homogeneous populations of the insect, we followed the methods of Hasan et al. (2012); insects were collected from different kinds of wheat grains and infected foods in different regions of Saudi Arabia, including Al-Ahsa, Riyadh, Al-Kharj and Hail, and were cultured in plastic containers with a capacity of 1000 ml in the incubator at a temperature of $2 \pm 27^{\circ}\text{C}$ and relative humidity 70%. The containers were covered with a muslin cloth with a rubber band attached, and the containers incubated in the incubator for two months. After that, the 5th instar larvae were separated for each of the treatment with three replications per treatment, and each replication consisted of 20 newly molted 5th instar larvae. The 5th instar larvae was chosen for the current study because, it was the gluttonous instar that attacked the stored wheat grains at 33.9% in three months (Musa & Dike, 2009).

Preparation of Plant Powders

The gum resin of the plants to be studied includes *Aloe vera* (L.), *Burm.f.*, *Astragalus sarcocolla* Dymoc, *Commefora myrrha* L and *Ferula assa-foetida*, which belonged to the families Liliaceae, Leguminosae, Burseraceae and Umbelliferae, respectively. The plants were obtained from herbal market Al-Kharj from a specialized taxonomist and washed with distilled water to remove the dust particles. Then, the plants were dried in the shade for 30 days, grinded by a high-voltage electric mill, and then sifted in sieved 60 mesh to obtain a very fine powder and kept in a glass containers in the refrigerator until use.

Bioassay Studies of Gum Resins Powders

To test toxic effects on the mortality of the 5th instar, the gum resin powders were incorporated into the 5th instar food of sterile wheat grains and were mixed well to ensure their full grain coverage. The concentrations 1%, 0.5%, 0.3%, and 0.1% "g/g" for each the gum resin powder were prepared separately and placed in sterile Petri dishes and each dish contained 10 g of the mentioned concentrations and 20 newly molted fifth instar larvae in three replicates. For the control treatment, the 5th instar was fed on non-treated wheat grains with the same number of larvae using the same method mentioned above. All treatments were carried out in containers with a capacity of 5 cm³, and the mortality percentage was recorded at 10th day of treatment. Mortality in controls was used to correct the mortality according to Abbott's formula (1925). To test the persistence or continuous effects of the gum resin powders on the larvae development and its pupation, the pupation was observed at 35th day, from the start of the first treatment and the number of pupa formed were recorded.

Statistical Analyses

All the data were statistically analysed using SPSS statistical analysis. One-way analysis of variance (ANOVA,

version 11) (Bluman, 2007) was used. The means were compared using the least significant difference (L.S.D) test.

RESULTS

A comparison of the mortality and pupation inhibition rates for the 5th instar larvae of *T. Granarium*, resulting from the concentrations applied for the gum resin powders *A. vera*, *A. sarcocolla*, *C. myrrha* and *F. assa-foetida* at 10th day of treatment are shown in Table and Table 2, and Table 3 shows that there were significant effects in mortality at 10th and 35th day respectively, of treatment, for all treatments, and the significant effect is due to the type of treatment (plant powder tested), not the concentration of the treatment effect; effects are considered significant at ($P < 0.05$).

Table 1: Larvicidal Activity of the Gum Resin Powders of *A. Vera*, *A. Sarcocolla*, *C. Myrrha* and *F.assa-Foetida* against 5th Instar Larvae of *Trogoderma Granarium* at 10 Day

Treatment	Mortality % \pm S.E.	95% Confidence Interval of the Mean
<i>A. vera</i>	0.13 \pm 0.085a	0.31
<i>A. sarcocolla</i>	0.00 \pm 0.00a	0
<i>C. myrrha</i>	0.13 \pm 0.085a	0.31
<i>F. assa-foetida</i>	1.44 \pm 0.540b	2.59
F	6.038	

Each value is a mean and standard error of three replicates. One-way mortality by type of treatment means within the same column, followed by the same letter is not significantly different at $P < 0.05$.

Table 2: Larvicidal Activity of Different Concentrations of the Gum Resin Powder of *F. Assa-Foetida* against 5th Instar Larvae of *Trogoderma Granarium* at 10 Day

Conc. w/w	Mortality % \pm S.E.	95% Confidence Interval of the Mean
0	0.0 \pm 0.0a	0
0.1	0.13 \pm 0.85a	0.31
0.3	0.38 \pm 0.315a	1.05
0.5	0.38 \pm 0.315a	1.05
1	0.81 \pm 0.421b	1.71
F	0.855	

Each value is a mean and standard error of three replicates. Means within the same column followed by the same letter are not significantly different at $P < 0.05$.

The results in Table 2 showed that, the gum resin powders that were tested at all concentrations applied did not cause a considerable mortality there were no changes in the data. The treatments were given 16.7%, 1% and 3.3% a mortality at the highest concentration for the treatment with the *F. assa-foetida*, *A. vera*, and *C. myrrha* gum resin powder, respectively, while the treatment with *A. sarcocolla* gum resin powder did not provide any mortality during this period, compared to 1.7% of the mortality in the control treatment. All the treatments at all the applied concentrations showed 100% mortality after 35 days of treatment. Similarly, the results increased the life span of the 5th instar larvae by 25 days more than the life span of the 5th instar larvae in the control experiment, which completed pupation in 10 days.

Table 3: Pupicidal Activity and Percent Inhibition of Pupation of the different Concentrations of the Gum Resin Powder of *F. Assa-Foetida* Against 5th Instar Larvae of *Trogoderma Granarium* at 35 Day

Conc. w/w	Mortality % \pm S.E.	95% Confidence Interval of the Mean	Percent Inhibition Pupation (%)
0.0	0.0 \pm 0.0a	0.0	2
0.1	0.38 \pm 0.15a	0.70	93.3
0.3	0.19 \pm 0.18a	0.59	100
0.5	0.0 \pm 0.00a	0.00	100

1	0.25 ± 0.19a	0.66	100
F	1.011		

Each value is a mean of standard error of three replicates. Means within the same column followed by the same letter are not significantly different at $P < 0.05$.

Table 3 shows the lowest inhibition pupation at 93.3%, which arose from a treatment with 0.1% gum resin powder *F. assa-foetida*; while treatment with the gum resin powders *A. sarcocolla*, *C. myrrha* and *A. vera* at all the applied concentrations inhibited pupation of 100%, at the same period of treatment for 35 days. These results are in contrast to the success of 98.3% emergence for adults in the control experiment, indicating the complete failure of adults to emerge in all treatments.

The results show that the tested plants have an effective toxicity and regulating effect on growth regulation for the 5th instar larvae. Before discussing this activity, it is useful to remember that the consideration of the time of recent and newly molted larvae of insects is the time that is more sensitive to the negative effects of growth regulators, especially those similar to the juvenile hormone JH action Gordon et al (1989) Additionally, Akunne et al. (2014) mention that the timing of the treatment plays important role in the expression of the growth regulators. With respect to the current result about the long duration of the 5th instar larvae, Jeyabalan et al. (2003) indicated that *Anopheles stephensi* larvae have a long life span after being treated with a *Pelargonium citrosa* extract. De Melo et al. (2015), Sagheer et al. (2014), and Shaaya et al. (1997) reported that the incorporation of the traditional medicine plant powders with stored products led to some bioactivity effects, such as larvicide, repellent, feeding deterrence, growth inhibition and morphological abnormalities.

For behavioural changes, the severity difference between the tested plants in a descending order was as follows: *F. assa-foetida* > *A. vera* > *C. myrrha* > *A. sarcocolla*. It appears that these changes indicate a strong repellent effect on the larvae by fully escaping from the wheat grains that were treated at different concentrations by all the treatments and attached to the lids of the experimental containers. This result was expected due to the offensive odours of these treatments, especially *F. assa-foetida* and *A. vera* powders. Bahrami et al. (2016) mention that the gum resin oil of *F. assa-foetida* may reduce the relative growth rate, feeding deterrence and other nutritional indices of the *Rhyzopertha dominica* (F.) adults after treating the wheat grain.

The odours of the gum resin powders of *A. vera*, *A. sarcocolla*, *C. myrrha* and *F. assa-foetida* led to a strong repellent effect against the 5th instar larvae and prevented them from feeding and disturbing the movement. Assuming disorder and neurotoxicity indicators, it is possible that, the modification of this toxicity leads to the penetration of some particles of these powders by the digestive or respiratory system of the larvae treated; this opinion is consistent with the study of Adedire and Ajayi (1996) who examined the toxicity of garlic powder and oil odours against *Callosobruchus maculatus*. Similarly, Najmizadeh et al. (2013) supports reported a field study in which the toxicity effect of gum resin oil *F. assa-foetida* on the *Thrips tabaci* pre-imago, which presented 96.7% mortality after 72 hours. Additionally, Bhalerao (2014) demonstrated that the mixed *Tribolium castaneum* food with 4 g/4 g of the *Aloe vera* gum extract has a toxicity efficiency of 100% mortality in adults and in fifth instar larvae after 72 hours of treatment.

According to the study by Kemabota et al. (2013), the 5th instar larvae have thick barbed hairs on their whole bodies, which provide them a lot of protection against the pesticide powders mixed with stored grains, in which they are scattered. Because of that, the highest possible treatment action tends to lead to odours. Assume there is a presence of a

neurotoxin in the treatment of 5th instar larvae through odours in which some of the biologically active compounds in the resin gum powder were tested. Notably, after that occurs, the movement disorder of the 5th instar larvae became more lethargic and weaker in the final days of exposure to the treatments. The results of Madhumathy et al. (2007) showed the potential of the *capsicum annum* on *Anopheles stephensi* and *Culex quinquefasciatus* larvae activity. which became inactive. Additionally, this lack of activity may be due to the hunger they suffered from treated food by those resin gum powders, because of their non-acceptance of the treated food in all treatments. Mahmoud et al. (2002) concluded that the refusal of the insect to consume treated food led them to lethargy and death. While Saxana (1987) and Rachid et al. (2006) reported that long-lasting hunger prevents development and coincides with the larvae having long periods of inactivity or feeding, the hunger also leads to irregularity in the moulting that occurred more than three times in all treatments and finally ended up failing to form the pupa cuticle before death. This sequence of events strongly suggests that the gum resin powders of the *A. vera*, *A. sarcocolla*, *C. myrrha* and *F. assa-foetida* possess a multi-force effect that begins with repellent, then starvation, and then nerve poison by disrupting the action of the active hormones, such as juvenile hormone (JH), ecdysone hormone (MH) and prothoracicotrophic hormone (PTTH). The action of the hormones are interrupted by continuing to secrete the JH longer than it is required, thereby leading to a failure to moult and form cuticles of pupa (pupation), and then leading to death before or during pupation. This process is called Arrested Development, and it occurs in the stages of insects treated with growth regulators; the effects of Arrested Development are similar to the JH hormone, as mentioned by Shantaram (1957), Hofmeister et al. (1988), and Gordon et al (1989) Moreover, Mahmoud et al. (2002) suggest that some plants are effective at causing instances that are repellent, antifeedant and hormonal for insect pest that may have a biological activity. Because of these effects, there are two possibilities for maintaining the effectiveness of *A. vera*, *A. sarcocolla*, *C. myrrha* and *F. assa-foetida* gum resin powders for up to 35 days.

The first is a chemical persistence for the powerful odours were that have not weakened over time, suggesting that their rich and varied chemical content is varied in biological activity and not limited to the insects in a single mechanism (Bakkali et al., 2008; Burt, 2004). The second is physical: due to the high content of the gum in the tested resins, it was observed that they adhere to each other and with the wheat grains. Additionally, this adhesion is considered to be an effective physical mechanism for preventing larvae from wandering in and between the grains, as well as stored grain containers. Instead, the adhesion forces larvae to remain on the surface of the containers or underneath them. According to these possibilities, the work of the gum resins is similar to the work of traditional inert powders. These results are in agreement with the work of Harnisch (1980), which suggested that the traditional inert powders depend on the physical work in protected stored grains and other products. Moreover, the inert powders may be more effective against the youngest instar larvae and adults via adhesion on their body, especially if the larvae do not have barbed hair.

CONCLUSIONS

This study revealed that, there are toxicity effects of gum resin powders, including *Aloe vera*, *Astragalus sarcocolla*, *Commefora myrrha* and *Ferula assa-foetida*, against the fifth instar larvae of *T. granarium*. This toxicity caused dysfunction in metamorphosis of the 5th instar larvae, and this was the first time this test has been ever conducted in this insect. Additionally, in a limited number of insects examined in previous studies, these gum resin powders are rich in growth regulatory characteristics as well as toxicity properties, against the larvae and pupa, and they have long-term persistence. These traits are the required characteristics of the insecticide used in the preventive and therapeutic control of the stored grains and products against a serious pest, such as *T. granarium* and can be safely used due to their plant origin

in the integrated insect control programmes.

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